

Historic, archived document

Do not assume content reflects current scientific knowledge, policies, or practices.

UNITED STATES DEPARTMENT OF AGRICULTURE



BULLETIN No. 319

Contribution from the Bureau of Animal Industry
A. D. MELVIN, Chief



Washington, D. C.

PROFESSIONAL PAPER.

January 10, 1916

FERMENTED MILKS.

By L. A. ROGERS,

Bacteriologist in Charge of Research Laboratories, Dairy Division.

CONTENTS.

	Page.		Page.
Introduction	1	The various forms of fermented milk.....	8
Therapeutic value of fermented milk.....	2	Bibliography.....	25
Food value of fermented milk.....	7		

INTRODUCTION.

Within recent years there has been a rapidly growing interest in the therapeutic value of buttermilk and other fermented milks, such as kefir, kumiss, and yogurt. This is seen in the increasing sale of buttermilk, in the large number of special preparations now offered for sale, and in the frequent discussion of this subject in popular and scientific publications. Buttermilk is not only consumed in large quantities as a beverage, but is recommended by physicians as a therapeutic agent in the treatment of intestinal disorders, and is in constant use in many hospitals.

It is the aim of this paper to give the reader a brief résumé of our present knowledge of this subject. The literature relating to fermented milks is already voluminous, and few persons, not even physicians, are so situated that this can be brought together and assimilated. It will be necessary for the benefit of those having a professional interest in the subject to include information of a somewhat technical nature.

All the more familiar fermented milks are the result of an acid fermentation in which the sugar of the milk is split up into lactic acid. This may be brought about by the presence in the milk of varieties of the common lactic-acid group of bacteria, or, as in the case of yogurt, by special organisms; or a yeast may be present, adding an alcoholic to the ordinary acid fermentation.

In many large cities special fermented milk preparations can be obtained under various trade names, such as zoulak, vitallac, yogurt, matzoon, bacillac, kefir, kumiss, and lacto-bacilline. These are all soured milks which have been introduced from southern Russia, Turkey, and neighboring countries. They are sold as freshly prepared milk, or in the form of tablets or powders in capsules which may be taken as such or used to ferment milk. These preparations have been widely advertised and are the subject of very positive statements in regard to the benefits derived from their use.

THERAPEUTIC VALUE OF FERMENTED MILK.

Fermented milks have been used ever since very early times, but it is only within very recent years that physicians have become interested in the possibilities of their use for therapeutic purposes. Within the past 20 years there has been an increasing number of papers in the medical journals on this subject, and at one time the widespread popular interest in fermented-milk therapy was reflected by the numerous magazine and newspaper articles on various phases of the subject. This interest was stimulated in a large measure by the work of Metchnikoff (58)¹ and his associates. His views, which are set forth in some detail in Chapter V, "Lactic acid as inhibiting intestinal putrefactions," of his book entitled "The Prolongation of Life," are looked upon by the more conservative investigators of this country as overdrawn and as unsupported by experimental evidence. In this book great stress is laid on the longevity of the people of certain countries in which fermented milks are an important part of the diet.

In considering evidence of this kind it should be remembered that many other things may contribute to the general health and vigor of the people and that these factors can not be excluded in drawing the conclusions. The people who habitually consume large quantities of fermented milk usually live a simple life, largely in the open air, and we have no means of knowing how much this may have contributed to the vigorous old age frequently observed among them.

The use of fermented milks as a therapeutic agent is based on the assumption that they are able to combat the so-called autointoxication caused by the undue accumulation in the body of toxic substances emanating from the intestinal tract. The theory of autointoxication may be stated briefly as follows: The digestive tract of the human being is at birth free from bacteria, but in various ways, chiefly through the food, many kinds of bacteria are introduced into the alimentary canal. In the intestines and particularly in the large intestine some of them find favorable conditions for growth and be-

¹ Figures in parentheses refer to the bibliography at the end of this bulletin.

come established there in large numbers. In the normally nourished infant the bacterial varieties are limited in number and for the most part consist of acid-forming types which by the active fermentation of the milk sugar furnished in large quantities in the food produce conditions under which bacteria of the putrefactive type are unable to multiply to any extent. The predominance of an acid fermentation in the large intestine produces an acid stool with a characteristic but comparatively unobjectionable odor. As the child gets older the variety of food is greater and the relative proportion of carbohydrates to protein is much reduced. In place of the acid fermentation there is a decomposition of the protein by other bacteria, intestinal gas is produced, and the stools become alkaline and frequently have a very objectionable odor. In the bacterial decomposition of the predigested protein it is supposed that products of a more or less toxic nature are produced. When the quantity of these products is relatively small they are disposed of through the normal channels and have no appreciable effect. If the excretory system fails to do its normal work, or if the protein decomposition is unusually active, toxic substances accumulate and the symptoms of autointoxication are produced. The production of toxic substances in abnormal amounts may be caused by a combination of circumstances promoting an unusual activity of putrefactive bacteria normally present, or it may be because the bacterial flora of the intestines changes and new bacteria are introduced.

The method of treating this condition by the use of sour milk is based on three conditions which may be stated as follows: (1) It assumes as correct the theory of the production of toxic substances in the intestine by the action of bacteria in quantities sufficient to cause the symptoms of autointoxication; (2) the putrefaction or fermentation through which these toxic substances are produced can be suppressed by other bacteria; and (3) the bacteria which it is proposed to use in suppressing the putrefactive bacteria may be introduced into the intestines and will be able to establish conditions there under which they will multiply and persist, while the objectionable types are driven out.

The standing of the theory of autointoxication mentioned under the first condition can not be discussed in detail in a paper of this nature. It may be said, however, that the question of autointoxication, in its broader sense, is not nearly so simple as it is stated here. It is at best only a theory, and much investigation of details will be necessary before its position can be determined.

The second condition is easily demonstrated, not only by scientific observation, but also by many instances in our daily life. Vinegar, which is used in pickle making, owes its preservative action to the

acetic acid produced by a bacterial fermentation; when milk sours spontaneously, the acid-forming bacteria develop acid so rapidly that in a short time all other bacteria are inhibited. Observations of this kind could be multiplied almost indefinitely. In fact, in the bacterial world, as among the higher plants in their natural state, there is a constant struggle for mastery in which the types best suited to their environments, or, perhaps more correctly, less sensitive to the unfavorable conditions which they themselves produce, gain the ascendancy and more or less completely suppress other forms.

The particular bacterium which it is proposed to use in suppressing the putrefactive bacteria of the intestines is the organism commonly known as *Bacillus bulgaricus*, or the Metchnikoff bacillus. It is characterized by its ability to form acid in exceptionally large amounts from sugars, particularly milk sugar.

When milk containing bacteria of this type is held under conditions favorable to its growth, the acid produced will inhibit other forms and the milk will eventually become a practically pure culture of the *Bacillus bulgaricus*. There can be no question that, under conditions favorable to its growth, this bacterium is able to suppress very effectively other kinds of bacteria, even many of those which produce an acid fermentation. This is well illustrated in the manufacture of cheese of the Emmental or Swiss type. Cheese made by this method from milk containing gas-forming bacteria will become filled with gas bubbles in the press. If a comparatively small amount of a culture of the *Bacillus bulgaricus* is added to this milk the high temperature at which the cheese is held promotes its vigorous development, and the gas formers are completely suppressed.

There is little doubt that if this organism could be established in the large intestine under conditions favorable to its growth it would soon produce a state of affairs which would at least inhibit the growth of the bacteria that usually decompose the proteins. The evidence that this takes place, even when large quantities of the bacteria are ingested, is by no means conclusive. On the one side the associates of Metchnikoff have produced considerable evidence to show that when *B. bulgaricus* is taken into the digestive system it becomes established in the intestines, where it persists for some time after the feeding ceases. Cohendy (12), who fed four patients for extended periods on milk curdled with *B. bulgaricus*, concluded that this organism was readily established in the intestines and that it persisted there for a considerable time after the subject had ceased to take fermented milk. This was said to be especially true if a diet containing suitable nourishment for the ingested organism was adopted. It is stated that the multiplication of these bacteria took

place in the upper two-thirds of the colon. The stools were acid or neutral.

The same writer in another paper (14) shows that intestinal putrefaction as indicated by the excretion of ethereal sulphates in the urine was materially reduced by the addition of a sour milk to the diet and that this reduction, which may reasonably be attributed to a disinfection of the large intestine, continued after the ingestion of sour milk was discontinued. This may be taken as an indication that the growth of the bacteria continued after their introduction ceased. This disinfecting action of the lactic-acid culture was not appreciably influenced by variations in the amount of sugar eaten, indicating that the ordinary diet contains sufficient sugar to support the growth of the lactic-acid bacteria in the intestine.

Belonovsky (3) arrived at somewhat similar results in experiments in which mice were fed a basic ration of sterilized grain and water. Mice which received in addition milk cultures of *B. bulgaricus* for one and one-half months showed this organism in the droppings 15 days after the last feeding. When the milk cultures were fed for four months *B. bulgaricus* was present in the droppings for four weeks after the last feeding. He states that the bacteria in the droppings, especially the gas-forming bacteria, were very much reduced by feeding *B. bulgaricus*, but were not affected by the addition of the basic diet of sterile milk or milk curdled with lactic acid.

Many experiments of a similar nature could be quoted, as well as clinical observations, tending to show that the ingestion of milk cultures of *B. bulgaricus* reduces or eliminates evidences of intestinal putrefaction. On the other hand, Herter (41) found that in the digestive tract of a monkey, killed after feeding for two weeks on milk soured with *B. bulgaricus*, this organism was abundant in the upper part of the small intestine only. In the lower part and in the large intestine *B. bulgaricus* was present in only moderate numbers as compared with other bacteria.

Rahe (70), in a recently published paper, maintains that the difference between the *B. bulgaricus* and certain acid-forming bacteria, which are known to occur normally in the intestines, is so slight that they can be distinguished only with difficulty, and he suggests that belief on the part of some investigators that *B. bulgaricus* becomes established in the intestines was caused by their inability to distinguish between these two types. His work tends to show that while the *B. bulgaricus* appears in the feces during the feeding, it persists for only a few days after the ingestion of cultures ceases.

The situation may, perhaps, be fairly summed up by saying that while there is no conclusive evidence that *B. bulgaricus* is able to establish itself in the intestines in such a way that other bacteria are driven out, it is undoubtedly true that in many cases marked improve-

ment has resulted from the ingestion of milk cultures made from it. It is by no means certain, however, that the results which have been obtained by the use of milk cultures have been attributable to any peculiar virtue in the organism itself. It has been held by some investigators that the intestinal flora may be radically changed by a fundamental change in the diet.

Distaso and Schiller (19) state that when rats were fed a diet of lactose and dextrine the heterogeneous intestinal flora was changed to one consisting almost exclusively of *Bacillus bifidus*, the characteristic acid-forming bacillus of the intestines. This is in accord with the earlier work of Herter and Kendall (43), who found that the nature of the bacterial flora of the intestines could be promptly and distinctly changed by a radical change from a diet high in protein to one in which carbohydrates predominated, or vice versa. A high-protein diet caused symptoms of intestinal putrefaction. A change to a carbohydrate diet resulted in a reduction of the putrefactive bacteria, an increase in the acid-forming bacteria, and the disappearance of the indications of autointoxication. Similar results were obtained in an investigation carried on by Rettger (71).

This work was very comprehensive, covering a long series of experiments with chickens and white rats, and the results if accepted will make it necessary to revise the commonly accepted views of the physiological actions of sour milk. Rettger found that when chicks were fed milk not only was the per cent of mortality materially reduced, but the rate of growth was greatly increased. Practically the same results were obtained whether sweet or sour milk was fed, and there was no appreciable difference in the results obtained by feeding milk soured by *Bacillus bulgaricus* and the common lactic forms. The probable explanation of this fact was found in the experiments with white rats, in which a study was made of the intestinal bacterial flora during the feeding experiments. It was found that when the rats were fed a diet which included milk, the usual mixed bacterial flora of the intestines was replaced almost completely by two types of bacteria which resemble the *Bacillus bulgaricus* very closely, especially in their physiological characteristics. Identical results were obtained when the milk was displaced in the diet by milk sugar.

The conclusion seems obvious. The bacteria of the high-acid type, which are apparently normally present in the intestines, are stimulated by the unusual amount of milk sugar furnished by the milk diet, and multiply to such an extent that the ordinary mixed flora is suppressed.

The beneficial effect of a sour-milk diet is attributable, perhaps, not so much to the bacteria contained in the milk as to the milk itself, which provides material for an acid fermentation in the intestines.

Milk is usually looked upon as a nitrogenous food, but it should be remembered that it contains about 5 per cent of lactose, a carbohydrate which seems to be peculiarly adapted to bacterial fermentation.

Aside from the possible therapeutic value of fermented milks, there can be no question that they are nutritious and refreshing and that their use should be encouraged for their food value.

FOOD VALUE OF FERMENTED MILK.

The high food value of milk is too generally recognized to need discussion here; fermented milks also have a high food value, except that in some cases the fat is partially or entirely removed. Otherwise the food value of the fermented milk differs little from that of the fresh milk from which it is made. Any increased digestibility of the fermented milk is due not so much to change in the chemical nature as to the fact that the casein is furnished in a precipitated and finely divided condition. In none of the fermented milks is there any material cleavage of the casein resembling the digestion in the stomach. The fat is practically unchanged, and a part only of the sugar is converted into acid, alcohol, or gas. In certain gastric troubles in which it is difficult to find any food that can be retained by the patient, fermented milks are frequently used with good results. Kefir and kumiss especially are used under such circumstances, as the stimulating action of the carbon dioxid which they contain is believed to aid in their digestion. To the physician the value of a highly nutritious food which can be digested when other foods are rejected is obvious.

There are many questions that should be very carefully considered before a fermented milk is introduced as an important part of the diet. As Herter (41) has pointed out in the admirable paper already cited, the addition of fermented milk to the diet may change very materially the ratio of protein to other classes of food. If the milk is taken in place of other food, the daily protein ration may be so reduced that intestinal putrefaction, which is dependent on the protein part of the food, is diminished. On the other hand, if milk is added to the usual food, the protein ratio may be increased rather than diminished. In many cases the condition of the mucous membranes will not permit the presence of organic acid, and soured milk can not be retained. It is also possible that symptoms of autointoxication are not caused by unusual bacterial activity in the intestine, but by functional failure of certain organs. This point could be determined only by a physician. It would be very unsafe to consume large quantities of milk, fermented or unfermented, under certain pathological conditions. In any case an important change in the diet should be made only upon the advice of a physician.

THE VARIOUS FORMS OF FERMENTED MILK.

If it is considered advisable to use cultures of acid-forming bacteria, the form in which they are taken becomes an important question. In large cities one usually has a choice of lactic-acid bacteria from several sources. Buttermilk is usually available, although it is not always of good quality. Sometimes kumiss or kefir can be obtained, and at the present time milk coagulated with the so-called Metchnikoff bacillus is sold as yogurt or matzoon and under various trade names.

CULTURES IN TABLET AND CAPSULE FORM.

In addition to these freshly prepared preparations several tablets or capsules purporting to be pure and active cultures of the *Bacillus bulgaricus* are now offered for sale. These are for use in fermenting

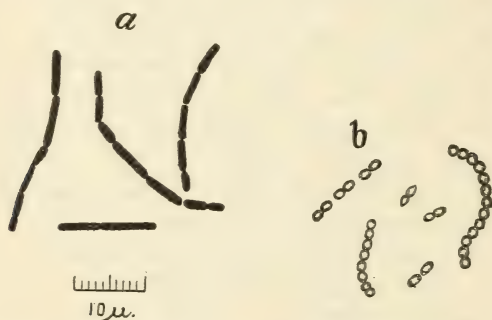


FIG. 1.—Organisms causing fermentation of milk.
a, *Bacillus bulgaricus*; b, lactic-acid bacteria.

milk or are to be taken directly in place of buttermilk or yogurt. Herschell (39) in his little book on the therapeutic uses of soured milks recommends the use of these preparations in preference to fermented milk, but it should be noted that he is very explicit in his statement that great care should be taken to determine the abundance and purity of the desired organism. *Bacillus bulgaricus* seems to be particularly sensitive to desiccation. When preparing the material in tablet form, therefore, a high percentage of the organisms is usually killed, whereas foreign organisms commonly retain their viability. Even when prepared with the best of care, such tablets lose their efficiency so quickly that unless such tablets are used within a time limit of efficiency guaranteed by the maker the results may be disappointing.

It is very easy to test the purity and activity of these dried cultures. Thoroughly pasteurize a small quantity (about half a pint) of milk by holding it, in a bottle plugged with cotton, at or near the boiling point for an hour or more. When this has cooled add two or three of the tablets and keep in a very warm place overnight. It should

not be below and may be a few degrees above blood heat. If in this time the milk has not curdled with a sharp, acid taste and without gas bubbles and whey there can be no reason for using these tablets except the possibility that they contain the active element of the culture which retards the growth of other bacteria. The evidence on this point is so inconclusive that it need not be considered in this connection.

All reliable manufacturers now place the date of manufacture on each package and state the time within which the tablets should be used.

BUTTERMILK.

Buttermilk, properly speaking, is the by-product resulting when milk or cream is churned for butter. It is the milk remaining after the fat which collects in granules is removed. If cream is churned when sweet the buttermilk does not differ from ordinary skimmed milk, but if it is churned when sour—the usual practice—the acidity is sufficient to coagulate the casein in the cream. In the churning process this curd is broken up into very fine particles. These curd particles settle very slowly, and if the buttermilk is agitated occasionally it will retain its milky appearance. When the buttermilk is allowed to stand undisturbed for several hours the curd particles sink to the bottom, leaving an opalescent whey at the top. At the present time a large part of the so-called “buttermilk” sold in cities is not buttermilk, properly speaking, since it is not made by churning cream, but is simply soured skimmed milk which has been churned or stirred in order to break up the curd. The same product is sold also under the name of “ripened milk.”

The souring of milk or cream is brought about by the activity of certain bacteria which form lactic acid by decomposing the milk sugar (lactose). The ability to form acid from lactose and other sugars is possessed by many kinds of bacteria, but is so characteristic of a certain group that they are commonly spoken of as the lactic-acid bacteria. (See Fig. 1, *b*.) These bacteria have been described as distinct species or varieties under many names. Among them may be mentioned *Bacterium guntheri*, *Bacillus acidi lactici*, and *Streptococcus lacticus*. In spite of the confusion in nomenclature it is evident that the term “lactic-acid bacteria” includes a fairly well-defined group of closely related varieties possessing in common several definite characters. Variations from the type in minor characters produce an almost infinite number of varieties. These variations may be in the ability to ferment different sugars, in the tendency to grow in chains, in the kind of flavor formed in milk, in the intensity of acid formation, and in the ability to produce pathological conditions in animals.

In many creameries the cream is allowed to sour spontaneously. In this case many bacteria other than the true lactic bacteria will take part in the acid formation, and in addition to lactic acid the buttermilk may contain in small quantities acetic, succinic, and formic acids, and sometimes traces of alcohol. The lactic bacteria form lactic acid, with only slight traces of other organic acids, no alcohol, and no gas. In well-managed creameries the acid fermentation is assisted and controlled to some extent by the use of a starter. This may be milk allowed to sour spontaneously, or buttermilk from the previous day's churning, but careful buttermakers build up starters from commercial cultures sold in the form of powders, tablets, or fluid cultures, as varieties of lactic-acid bacteria selected with special reference to the production of a desirable flavor in butter. The buttermaker puts this culture into about a quart of milk which has been steamed for an hour or more to reduce the bacteria to the lowest possible number. After standing overnight the milk will usually be curdled, but gas bubbles and other evidences of contamination may be observed. A small portion of this milk is transferred to another bottle of milk prepared as before, and this process is continued until the acid fermentation has become sufficiently active to eliminate the contaminating bacteria, and the milk curdles with a clean, acid taste and without signs of gas or "wheying off." This small starter, or "mother starter," is carried along indefinitely by daily transfers to freshly steamed milk. If reasonable precautions are taken to prevent contamination after a thorough heating of the milk, this culture will remain pure and vigorous for an indefinite time.

To prepare the starter actually used in ripening the cream, a larger lot of milk—25 to 50 gallons or more, according to the amount of cream—is heated for an hour or more. This is usually done in a special apparatus (sold by creamery supply houses) which consists of a large can inclosed on the sides and bottom by a steam jacket and fitted with a belt-driven stirrer. Milk either skimmed or unskimmed is heated by turning steam into the jacket; during the heating the milk is stirred constantly. After the pasteurization is completed cold water is run into the jacket and the milk cooled to about 24–27° C. (75.2–80.6° F.). A bottle of the mother starter is added and the can is covered and allowed to stand overnight. This gives a large and active pure culture of lactic-acid bacteria to start the acid formation in the cream. Better results are obtained if the cream is first pasteurized.

When lactic-acid bacteria grow in milk the lactose is converted into lactic acid with slight traces of certain other organic acids. This acid breaks up the combination of calcium phosphate and casein which holds the casein in solution, and the casein is precipitated as a firm, jellylike mass. When this occurs in cream the fat globules are en-

tangled in the precipitated casein. In the process of churning the casein is broken into fine particles and the fat globules are collected into large granules that float on the top of the buttermilk. Buttermilk, then, is the water of the milk holding the sugar, acids, ash, and other soluble constituents in solution and the finely divided particles of precipitated casein in suspension. The amount of fat in the buttermilk depends on the completeness with which the fat is removed in the churning. Even with the best methods a little of the fat in the form of very small globules remains in the buttermilk. On standing, the suspended casein settles slowly to the bottom.

The composition of an average buttermilk is about as follows:¹

	Per cent.
Fat.....	0.5
Casein.....	2.4
Albumin.....	.6
Lactose.....	5.3
Ash.....	.7
Total solids.....	9.5

Chemically, buttermilk differs but little from skim milk. Only a slight rearrangement is necessary to bring about the physical change in the casein. If the milk has been pasteurized at a high temperature, the albumin is precipitated and the larger part lost. A small part—less than one-fifth—of the milk sugar is converted into acid. This acid combines with the ash constituents, probably converting the triphosphates into diphosphates and monophosphates and the diphosphates into monophosphates. It is obviously not necessary to make butter in order to secure a perfect substitute for buttermilk. Soured skim milk has all the chemical properties of buttermilk, and if it is thoroughly agitated in order to break up the curd it agrees in appearance and flavor with buttermilk obtained by churning cream.

In making buttermilk from milk the same procedure should be followed as in making a starter for cream ripening. A good, clean-flavored mother starter should be carried along with every possible precaution to prevent contamination. Good commercial cultures can be obtained, but if it is not convenient to use one of these a natural starter should be secured. For this purpose the following procedure may be followed:

(1) Select milk from several sources; put about 1 pint of each into clean glass jars or bottles and allow them to stand in a warm place until the milk is curdled.

(2) When this occurs put about 1 pint of milk into each of an equal number of bottles and hold in steam or boiling water for one-half hour.

¹ Vermont Agricultural Experiment Station. Annual Report, 1891, p. 119.

(3) When these bottles of milk are cooled, transfer about 1 teaspoonful of milk from each of the bottles of sour milk obtained in (1) to one of the bottles of heated and cooled milk.

(4) Allow these samples to curdle and repeat the process until one sample is obtained which curdles in at least 8 or 10 hours with a smooth curd free from whey and gas bubbles and with a pleasant, acid taste.

Gas bubbles, or the separation from the curd of a milky or straw-colored whey, show that the lactic-acid bacteria are still mixed with other kinds. Considerable variation in flavor can be found in different cultures, and care should be exercised to select one that gives a clean and sharp taste.

(5) Propagate this culture in the same way from day to day. The amount of this mother starter which should be carried will depend somewhat on the amount of buttermilk to be made. One quart should be enough for 20 to 30 gallons.

(6) (a) Add the mother starter to the milk to be used for buttermilk, or (b) pasteurize the milk in a continuous pasteurizer at 180° to 185° F. (82° to 85° C.), or preferably hold the milk in water-jacketed vats or cans at 180° F. (82° C.) for 30 minutes to an hour; cool to about 70° F. (21.1° C.) and add the mother starter. The most desirable temperature for this fermentation is 70 to 75° F. (21.1° to 24° C.).

(7) When this milk has curdled, cool it at once to about 50° F. and churn thoroughly to break the curd into fine particles.

The buttermilk should be smooth, free from lumps, and show a separation of whey and curd only on long standing.

Milk to be used for making buttermilk should be fresh and clean flavored. Good buttermilk can not be made from milk that is tainted or too old to be used for other purposes. Skimmed, partly skimmed, or whole milk, as desired, may be used.

A more nearly uniform product is secured if the milk is pasteurized. The scorched taste which results from pasteurization at a high temperature is not objectionable, as it is obscured by the acidity of the soured milk. The time of the inoculation may be arranged to suit the convenience of the maker and can be determined by experience in each individual case. Using the same culture and holding the temperature uniform, the amount of the starter can be adjusted to bring the acidity to the curdling point at any definite time within narrow limits. The temperature of the milk should be between 21° and 24° C. (70° and 75° F.). More rapid development of acid can be obtained at higher temperatures, but at the lower temperatures the lactic-acid bacteria are more successful in checking the growth of digesting and gas-forming bacteria. At lower temperatures and with a slower development of acid the casein is precipitated in a finer and more friable

curd than at temperatures inducing a more rapid acid production. As soon as a fine curd has been formed the milk should be cooled promptly to below 50° F. to prevent the contraction and toughening of the curd.

Buttermilk made in the usual way as a by-product of buttermaking, and especially buttermilk obtained by churning pasteurized cream, is improved by mixing with it about 10 per cent of a skim-milk culture of the *Bacillus bulgaricus*. Directions for the preparation of this culture will be found on page 23.

This culture not only gives the buttermilk a sharper and more agreeable flavor, but on account of its viscous nature it gives it a smoother texture and prevents the separation of the curd from the whey. Detailed directions for the preparation of buttermilk by this method may be found in a circular of the Illinois Agricultural Experiment Station (52).

MAKING BUTTERMILK IN THE HOME.

A more nearly uniform product can be obtained if it is made on a large scale, and if good buttermilk can be purchased from a reliable milk dealer at a reasonable price it is not advisable to attempt to make it on a small scale. However, it is possible to make buttermilk in the home by following in a small way the directions for making buttermilk on a commercial scale. It is necessary first to secure a culture or starter, which is merely milk containing the lactic-acid or sour-milk bacteria free or very nearly free from other kinds. These bacteria are present in any normal milk, and it is only necessary to provide conditions favoring their growth to obtain them in a state of purity.

This may be done by following the directions on page 11 for obtaining cultures for making buttermilk on a large scale. When the culture is obtained it will not be necessary to carry a small culture to inoculate a larger amount.

When the culture is obtained proceed as follows:

(1) Heat 1 quart of milk, which may be skimmed, in a double boiler for at least one-half hour.

(2) Allow the milk to cool to about 75° or 80° F.

At this temperature the outside of the container will feel warm to the hand. Add one teaspoonful of the fresh culture, transfer to a bottle or covered fruit jar, and put away in a warm place. One of the vacuum-jacketed bottles will be found very convenient for this purpose, because the milk can be held at a nearly constant temperature favorable to the growth of the lactic-acid bacteria.

(3) On the following day shake the bottle thoroughly to break up the curd and put the product on ice to cool.

(4) Repeat the process, using a teaspoonful of the freshly curdled milk to inoculate the heated and cooled milk.

Buttermakers in the Northwest make a very refreshing and nutritious drink by adding sugar and lemons to buttermilk. As the casein is already precipitated, the acid juice of the lemon has no effect. Slightly more sugar and lemon juice are necessary than in making ordinary lemonade, and the mixture should be well iced.

KEFIR.

Fermented milks have evidently been extensively used for many centuries by the people of southern Russia, Turkey, the Balkan countries, and their neighbors. The natives have no records and few traditions of the origin of the milks they use, and it is probable that their preparation and use developed gradually by accident and cumulative experience. One of the first of the fermented milks known to Europeans was the kefir, made from the milk of sheep, goats, or cows in the Caucasus Mountains and neighboring regions. Kefir differs from most of the fermented milks of the Mediterranean countries in that it is made from a dried preparation and contains considerable quantities of alcohol and gas. Kefir is made by many tribes under varying names, as "hippe," "kepi," "khapon," "kephir," "kiaphir," and "kaphir," all of which are said to come from a common root signifying a pleasant or agreeable taste.

For a large part of their food the mountaineers of the Caucasus depend on kefir, which they prepare in leather bottles made from the skins of goats. In the summer the skins are hung outdoors, either in the sun or in the shade, according to the weather, but in winter they are kept in the house. The bags are usually hung near a doorway, where they may be frequently shaken or kicked by each passer-by. Fresh milk is added as the kefir is taken out, and the fermentation continues. Made and propagated in this way, foreign bacteria become mingled with the essential bacteria of the grains, and abnormal and frequently disagreeable flavors result. When the milk is drawn off, in order to prevent the escape of gas, a string is first tied around the neck of the leather bottle, so that the small part wanted for use is held between the stricture and the opening. In the villages and the low country kefir is made in open earthen or wooden vessels, and most of the gas escapes.

Small, yellowish, convoluted masses are observed in kefir, which are called seeds or "grains." These grains consist of a central filament of two parts, of which the outer spreads out, forming the convoluted polyp-like exterior. These parts are built up one upon another, giving the large grains a coral-like appearance. The central part is made up of a mass of bacterial threads. In the outer layer

yeast cells are found mingled with the bacteria. When the grains are added to milk they swell and increase in size by forming new grains. At the beginning of the fermentation they settle to the bottom, but in a short time they are carried to the surface by attached bubbles of gas. If the fermentation is active, a thick layer will be formed on the surface, but on shaking or stirring this layer settles again to the bottom.

The biology of kefir was studied by Kern (45) in 1881, but, owing to the faulty technique of that day, his descriptions are evidently erroneous.

Freudenreich (25) describes four organisms that he isolated from kefir grains. One of these was a yeast which he designated *Saccharomyces kefir*; this he found to grow best at 22° C. (72° F.), but not at all at 35° C. (95° F.). It ferments maltose and cane sugar, but not lactose. It gives a peculiar flavor to milk, but causes no fermentation. The cells are oval, 3 to 5 microns by 2 to 3 microns. It is not identical with the ordinary beer yeasts. Two of the organisms were of the lactic-acid bacteria type, but differed from them in forming gas in lactose media. The most interesting of the organisms described is a long, slender bacillus corresponding to one described by Kern as *Dispora caucasica* and to which Freudenreich gave the name *Bacillus causicus*. In morphology, failure to grow on ordinary laboratory media, and in high-acid production in milk, this bacillus resembles very closely the bacillus mentioned later, in connection with yogurt, as *Bacillus bulgaricus*. If Freudenreich's description is accurate, *B. causicus* differs from *B. bulgaricus* by forming gas from lactose and in being feebly motile. Gas was formed slowly at 35° C. and still more slowly at 22° C. (72° F.). No one of these organisms alone produced kefir, but when the four together were grown in milk typical kefir was produced on the first or second transfer.

According to the investigations of Nikolaiewa (64), three organisms are always present in the fermented milk. One of these, *Bacterium causicum*, which forms the filament of the grain, is evidently identical with Freudenreich's *Bacillus causicus*. This investigator considers this bacterium, with a torula yeast fermenting lactose, dextrose, and cane sugar, as essential to the production of kefir. Other bacteria and yeasts are found in the grains and the fermented milk, but they are looked upon as contamination.

It is probable that kefir is produced under different circumstances by different organisms. Any combination of bacteria or of bacteria and yeasts that will produce a lactic-acid and a mild alcoholic fermentation in milk will make kefir, although to secure the most desirable flavor certain organisms may be essential.

Hammarsten (31) shows in the following table the changes brought about in cow's milk by this fermentation:

Chemical analysis of kefir.

	2 days old.	4 days old.	6 days old.
Casein.....	2.570	2.586	2.564
Lactalbumin.....	.425	.405	.390
Peptones.....	.071	.089	.120
Lactose.....	3.700	2.238	1.670
Fat.....	8.619	3.630	3.626
Ash.....	.641	.624	.630
Lactic acid.....	.665	.832	.900
Alcohol.....	.230	.810	1.100

It will be observed that the changes were confined almost entirely to the lactose and its by-products. The casein remained unchanged and the increase in the peptones was insignificant. The lactalbumin decreased slightly. The casein of kefir is, according to this chemist, not especially soluble, but may be more easily digestible because of its finely divided condition. The lactose diminished appreciably, and there was a corresponding augmentation of alcohol and lactic acid. A certain part of the lactose is consumed in the formation of carbon dioxid gas not included in this analysis.

The following directions are given for making kefir when the grains are available: The dry grains are softened by soaking in warm water, which should be changed several times. When the grains rise to the surface and become white and gelatinous they are ready for use. One part of these grains is used to three parts of milk which has been thoroughly heated to destroy the bacteria already present. The bottles in which the milk and grains are placed should not be stoppered but should be protected from the dust by cloths, inverted cups, or plugs of cotton. They are held at a temperature at or near 14° to 16° C. (57° to 60° F.), and stirred or shaken frequently. After eight to ten hours the milk is strained through cloth and put into tightly stopped bottles at the same temperature as before. The bottles should be shaken every few hours to prevent the formation of lumps of precipitated casein. The kefir is ready for use at the end of 24 hours; if held longer than this it is advisable to keep it on ice to check the fermentation. The temperature at which the milk is fermented is important in controlling the relative amounts of alcohol and lactic acid. At higher temperatures the percentage of alcohol is increased, while as the temperature is lowered the alcoholic fermentation diminishes and the quantity of lactic acid formed is greater. After the fermentation is once started the grains may be discarded and new kefir made by adding one part of the fermented milk to three or four of fresh milk. In

order to remove the grains the kefir should be strained through cheese cloth, and after thorough washing to remove the curd the grains may be dried by exposure to the sun on pieces of blotting paper. In this condition they are said to retain their vitality for several years, although many of the yeasts in the outer part of the grain are killed by the desiccation. It may be necessary to break up the grains with the fingers. When in the wet stage they should not be larger than a walnut.

Kefir grains can not ordinarily be obtained in this country, but a good imitation of kefir can be made by carrying on simultaneously in sealed bottles an alcoholic and a lactic fermentation. Better results can be obtained by inducing the alcoholic fermentation in buttermilk. In this way it is possible to avoid much of the trouble from the formation of lumps of curd. If buttermilk is made for this purpose from whole or skimmed milk, careful attention should be given to the time of curdling and the breaking up of the curd. This is essential to a smooth, creamy kefir. Ordinary bread yeast may be used for the alcoholic fermentation, but as this yeast does not ferment lactose it is necessary to add cane sugar to the milk.

(1) Obtain buttermilk from a dealer, or prepare it as directed on page 11.

(2) Prepare the yeast by adding a half teaspoonful of sugar to a 6-ounce or 8-ounce bottle of boiled and cooled water. Add half a yeast cake to this sugar solution and set in a warm place overnight. This will give an active culture of the yeast and obviate the necessity of adding the yeast cake directly to the milk. This yeast culture should be ready at the time the buttermilk is received or, if made at home, at the time it is curdled.

(3) Add 1 to $1\frac{1}{2}$ per cent of sugar to the buttermilk.

On the quantity of sugar added to the buttermilk will depend the extent of the alcoholic fermentation. Theoretically about one-half of the sugar fermented may be converted into alcohol; that is, milk to which 1 per cent of cane sugar has been added may contain after the fermentation one-half of 1 per cent of alcohol. The quantity of sugar added should be governed by the amount of carbon dioxide it is desired to have in the finished product. This should be sufficient to make the kefir distinctly effervescent and impart to it the peculiar, sharp taste of charged water, but should not be developed enough to blow the fluid out of the bottles when the stoppers are removed. Experience shows that 1 to $1\frac{1}{2}$ per cent of sugar will give the right amount of gas. This may be approximated by adding sugar in the proportion of two even teaspoonfuls of sugar to each pint of milk.

Having the buttermilk and the yeast culture ready, dissolve the sugar in the buttermilk.

(4) Add the yeast culture to the buttermilk in the proportion of one teaspoonful to a quart of buttermilk.

(5) Mix thoroughly and bottle. The bottles should be very strong, as sufficient gas pressure is sometimes generated to break ordinary bottles; the heavy bottles used for ginger ale or other carbonated drinks answer this purpose very well. They should be carefully cleaned and boiled or steamed before filling; fill them full and stopper tightly, wiring or tying the stoppers securely in place.

(6) Place in a cool place to ferment.

If the fermentation is too active the kefir will have a yeasty taste and the curd is likely to become lumpy and filled with large gas bubbles. A temperature of 18° C. (65° F.) to 21° C. (70° F.) will be found satisfactory for kefir which is to be used on the third or fourth day. The floor of a cool cellar is a convenient place to ferment kefir made in the home. The bottles should be shaken as often as may be necessary to keep the curd in a finely divided condition. The finished product should be smooth and creamy, effervesce rapidly when poured from the bottle, and have the pleasant, acid taste of buttermilk, with the added sharpness caused by the gas and the trace of alcohol. Kefir 2 or 3 days old may have a yeasty taste, but if it has been properly made this will disappear as the fermentation of the sugar nears completion; made under these conditions it should be used when 3 to 5 days old, but if put on ice it may be held for a week or even longer.

KUMISS.

The missionary monks and other wanderers who first penetrated the undulating, treeless plains of European Russia and central and southwestern Asia brought back descriptions of a fermented drink which in the light of more recent investigations is easily recognized as kumiss. These vast prairies are inhabited by tribes of nomads who live in tents or squalid huts in the winter and wander during the summer, seeking pasture for their horses, their herds of cattle, or flocks of sheep. They are all horsemen, and by a process of selection in which they have probably played only a passive part have developed an exceptionally hardy race of horses. The mares give much more than the ordinary amount of milk, which constitutes almost the entire food of the people during the summer. This is never used in the fresh condition, but is fermented to make kumiss. Unlike kefir, there is no dried "ferment," "seeds," or "grains" with which the fermentation of the mare's milk is started. It is the practice of the natives, when it becomes necessary to establish the fermentation anew, to add to milk some fermenting or decaying matter, such as a piece of flesh, tendon, or vegetable matter. Whatever the material used to supply the essential organisms, it is evident that the milk is so cared

for that a combination of an acid and an alcoholic fermentation is favored and the necessary bacteria and yeast are soon established. No doubt the change in the milk is produced under different circumstances by different combinations of bacteria and yeast, and there are usually present various contaminating organisms which are detrimental or at least are not essential to the production of the kumiss. Native kumiss makers lay great stress on the quality of the milk, the breed of the mares, and the condition of the pastures; but it is probable that their troubles ascribed to variations in these conditions are more likely attributable to imperfectly controlled bacteriological factors.

There was at one time much interest in kumiss as a therapeutic agent in the treatment of tuberculosis, and sanatoria were established in Russia where invalids could get this treatment. It is probable that the benefits, real or imaginary, derived from it came more from the general methods, which correspond somewhat to present practices, than from the action of kumiss.

Mare's milk is lower in nutritive value than cow's milk, as the following table, taken from Richmond's Dairy Chemistry, shows:

Average composition of cow's milk and mare's milk.

	Water.	Fat.	Sugar.	Casein.	Albu- min.	Ash.
	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>
Cow.....	87.10	3.90	4.75	3.00	0.40	0.75
Mare.....	90.06	1.09	6.65	1.89		.31

The composition of kumiss varies somewhat with the age, the rapidity of the fermentation, and the nature and extent of contamination with extraneous organisms. The following analysis is taken from Richmond's Dairy Chemistry (p. 241):

Composition of kumiss made from mare's milk.

	1 day old.	8 days old.	22 days old.
	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>
Water.....	91.43	92.12	92.07
Alcohol.....	2.67	2.93	2.98
Lactic acid.....	.77	1.08	1.27
Sugar.....	1.63	.50	.23
Casein.....	.77	.85	.83
Albumin.....	.25	.27	.24
Albumose.....	.98	.76	.77
Fat.....	1.16	1.12	1.30
Ash.....	.35	.35	.35

It will be observed that this fermentation produces no changes that could be expected to increase appreciably the digestibility of the

nitrogenous part of the milk except the possible advantage of a finely divided curd. Mare's milk differs from cow's milk in giving with rennet a softer, more friable curd, but it is not certain that this property would increase the value of kumiss.

Kumiss is often made and offered for sale in this country, but as this is usually made from cow's milk, it is, more correctly, kefir.

YOGURT.

In passing to a consideration of the fermented milks used by the people of the countries bordering on the eastern end of the Mediterranean we find a preparation very distinct from that of the Caucasus and the Russian steppes. Kefir and kumiss are limpid, mildly acid, and distinctly alcoholic; but the yogurt, yahourth, or jugurt of the Turks, the kissélo mléko of the Balkan people, the mazun of Armenia, the gioddù of Sardinia, the dadhi of India, and the leben or leben raib of Egypt, are all thick-curdled milks, decidedly acid, and with very little or no alcohol. The method of preparation is also quite different. Goat's, buffalo's, or cow's milk may be used. This is usually boiled and sometimes is reduced by evaporation to one-half its original volume. In the latter case it is not used as a drink, but is eaten, frequently with the addition of bread, dates, or other food.

A portion of the previously fermented milk is used to ferment the fresh milk. Unlike kefir, there are no "seeds" through which the fermentation can be transmitted, but the essential organism is sometimes preserved by drying the fermented milk and reducing the dry material to a powder. This constitutes the "podkwassa," or "maya." The organism giving these milks their distinctive character is evidently identical in them all, or, more properly speaking, may be any one of the several varieties of a distinct and closely related group. On account of its peculiarities, some of which are exceptional and striking, and the importance recently attached to it by the discussions both in the scientific and the popular press, a brief résumé of its characteristics is given:

This bacterium was probably first observed by Kern (45), who incorrectly designated it *Dispora caucasicum*. His description, however, is so limited that it is impossible to attach the name he proposes to any particular organism. Later Beyerinck (6), under the name *Bacterium caucasicum*, and Freudenreich (25), as *Bacillus caucasicus*, described organisms isolated from kefir which agree in their essential features with those obtained from yogurt. More recently Rist and Khoury (72) isolated from Egyptian leben two bacilli to which they gave the names *Strepto-bacillus lebensis* and *Bacillus lebenis*. Grigoroff (29) and Cohendy (13) isolated similar organisms from Bulgarian fermented milk. These various bacteria are

undoubtedly nearly or quite identical and all are included under the name *Bacillus bulgaricus*, now generally adopted. More strict adherence to the commonly accepted rules of bacteriological nomenclature would retain the name *Bacterium caucasicum* proposed by Beyerinck. Recent work by Hastings (32) and by Heinemann and Hefferan (36) indicates that this bacterium is not peculiar to the eastern fermented milks, but is widely distributed, having been isolated from milk, soil, saliva, feces, and various soured foods. White and Avery (82) believe that this bacterium is the representative of a group of closely related bacteria which they divide into two types on the basis of their activity in milk and the nature of the lactic acid formed. The characteristics of the typical culture may be summarized as follows:

Morphology.—Slender rods 2 microns to 6 or 8 microns in length, breadth usually about 1 micron, flagella and spores absent. Long chains frequently occur and apparently vary with different strains and conditions; pseudobranching has been observed. Very long threads without apparent division are frequently observed in old cultures. Living cells are gram positive; dead cells are gram negative.

Growth on artificial media.—One of the most striking features is its inability to grow on ordinary media. It grows on whey, malt, and slowly on whey agar and certain specially prepared media. The colonies on whey agar are masses of tangled threads resembling colonies of the anthrax bacillus. Gelatin is not liquefied.

Relation to oxygen.—Most varieties grow equally well in the presence or absence of oxygen.

Temperature relations.—The maximum temperature is near 45° C. (113° F.). The minimum growth temperature varies with different members of the group, but it is always comparatively high. Most varieties grow very slowly at 25° C. (77° F.), but some grow at 20° C. (68° F.). Hastings and Hammer (33) state that at 20° C. (68° F.) it forms 4 per cent acid in milk as compared with a maximum of 3 per cent at 37° C. (98° F.). According to White and Avery (82) it is killed by an exposure of 15 minutes at 60° C. (140° F.).

Fermentation of sugars.—Many of the sugars are fermented, but statements of different workers are conflicting. It is probable that this property varies in different varieties.

Milk.—The action of this organism on milk distinguishes it from all other known bacteria. At the optimum temperature milk is curdled in a few hours with a rather soft curd, frequently somewhat slimy, which ordinarily does not separate from the whey even on long standing. In 24 hours the milk may show acidity equivalent to

nearly 2 per cent of lactic acid, and on standing several days this may become about 3 per cent. The most active of the ordinary lactic-acid bacteria seldom exceed 1 per cent lactic acid. The more active type of *Bacterium caucasicum* forms the inactive lactic acid, while the levorotatory acid is produced by the type forming acid more slowly. Small amounts of other organic acids and traces of alcohol are formed.

This bacterium is evidently the *essential* organism of yogurt, matzoon, ceiddu, leben, and similar fermented milks. Other bacteria are always present, some of them habitually and others only occasionally. Some of these may have an influence on the flavor, while others are inert. It is probable that there are none, with the exception of *Bact. caucasicum*, that can not be replaced by other species without appreciably affecting the results. Doubtless slightly different varieties of fermented milk have developed in different localities owing to different combinations of bacteria or of bacteria and yeasts. The Egyptian leben is reported to contain alcohol, but not in quantities sufficient to produce an effervescence such as is observed in kefir or kumiss. One of the ordinary lactic-acid bacteria seems to be always present with the *Bact. caucasicum*, and it is probable that if it is not essential it is of some assistance in starting the lactic fermentation and, especially if the temperature is low, in suppressing contamination before the *Bact. caucasicum* has time to develop sufficient acid to check extraneous bacteria.

Hastings and Hammer (33) could not detect evidences of proteolytic enzymes by the usual tests, but found in old-milk cultures a distinct peptonization of the casein which was not traceable to the action of the acid. This change is so slow and so small that it can not be considered as having any influence on the digestibility of the milk. Otherwise the only changes in the milk constituents are in the conversion of the sugar to lactic acid and very small amounts of volatile acids and traces of alcohol.

"Yogurt buttermilk" is now sold in several cities, and the growing demand will doubtless soon extend its manufacture more generally. In making yogurt in this country better results are obtained by using with the *Bact. caucasicum* a culture of an ordinary lactic-acid organism such as is used in making buttermilk. *Bact. caucasicum* growing alone in milk forms usually a rather slimy, tenacious curd which can not be broken up into the smooth, creamy condition essential to a good buttermilk. If this organism is grown in combination with the ordinary lactic-acid organism, a more friable curd is obtained, and the sliminess is not so evident. The two organisms can be carried in mixed culture only with great difficulty, as the high acid soon suppresses the ordinary form. The most satisfactory results can be ob-

tained by making buttermilk in the ordinary way and churning it with an equal quantity of milk curdled with the yogurt organism. This procedure gives the desirable texture of buttermilk and a distinctive flavor.

If a culture can be obtained, yogurt can be made in the home. If a reasonably active dry or fluid culture can be obtained, the following procedure should be satisfactory:

(1) Heat one-half pint of milk in a double boiler, holding it one-half hour after the water begins to boil.

(2) Cool this milk to about 100° F. (about blood heat). At this temperature the container will feel warm, but not hot, to the touch.

(3) Add a considerable quantity of the culture to this milk. If it is in the form of tablets three or four should be used.

(4) Transfer the milk to a bottle or fruit jar—or, better still, a vacuum-insulated bottle—which has been rinsed with boiling water, and hold overnight in a warm place. Good results may be obtained by placing the bottle or jar, containing the milk, in a dish of water warmed to about 100° F. The most favorable temperature for the fermentation is at or a little below blood heat. At a little higher temperature the organism grows faster, but the curd is likely to separate from the whey as a tough mass. At a lower temperature the growth may be so slow that other bacteria gain the ascendancy. By the following morning the milk should be curdled with a thick, somewhat stringy curd with a sharp, acid taste.

(5) Heat 1 pint to 1 quart of milk as in (1), cool and add 1 teaspoonful of the curdled milk obtained in (4).

Hold this milk as before, and when it has curdled break up the curd by shaking vigorously in a fruit jar.

This process may be repeated so long as the curdled milk has a smooth, acid curd free from undesirable flavors and particularly the yeasty flavor and odor characteristic of bread dough. The so-called *Bacillus bulgaricus*, under favorable circumstances, will suppress other bacteria by its vigorous acid formation, but yeasts are favored by the acid condition of the milk and sooner or later make their appearance. Every precaution should be taken to protect the milk from exposure to the air and to sterilize all utensils with boiling water. When evidences of yeast contamination appear it is best to start with a fresh culture.

Yogurt may be made more palatable by adding to two parts of the yogurt one part of cold water, or, better still, cold-charged water, which can be bought in siphons at drug stores. Sugar and lemon juice or other fruit flavor, or chocolate sirup may also be used for this purpose. The sugar should be added in the form of a sirup, as granulated sugar dissolves very slowly in the cold yogurt.

In making yogurt on a large scale the process is not essentially different except that it is advisable to carry a small culture, about 1 quart, to inoculate the milk to be made into buttermilk. Every precaution should be taken to maintain the purity of the culture. It is advisable to carry duplicate cultures independently so that a good one will always be available.

Expensive outfits for making fermented milks are on the market, but while they may be convenient they are by no means essential. For the smaller dairy the following procedure will probably be found satisfactory:

(1) Propagate a small culture from day to day as indicated in the directions given above.

(2) Carry in a similar way a culture of the ordinary sour-milk organism, which may be obtained from many of the commercial laboratories.

(3) Thoroughly pasteurize the milk to be fermented. If a small quantity—5 to 10 gallons, for instance—is to be made, it may be done by holding a can of milk in a tub or vat of water heated by a steam hose. If a larger quantity is made, one of the starter cans used in creameries will be found convenient. These are essentially cylindrical vats with mechanical stirrers and a jacket which can be filled with steam for heating or water for cooling. The milk should be held at a temperature of at least 180° F. for not less than 30 minutes.

(4) Cool the milk to about 100° F. Draw off one-half and inoculate it with the culture obtained in (2). Inoculate the remaining half with the bulgaricus culture obtained in (1). The amount to be added will depend on the quantity of milk to be fermented, the time at which it is desired to have it curdled, and the temperature maintained during the fermentation. This can best be determined by experience. One pint should be sufficient for any amount between 10 and 20 gallons.

(5) The milk inoculated with (2) may be held at ordinary room temperature. Precautions must be taken to hold that part inoculated with the bulgaricus culture at a temperature of 90° to 100° F. for several hours. If the milk is in cans it may be set in a tub of warm water. A large volume of milk in a warm room will maintain the proper temperature.

If one is unable to hold the milk at the desired temperature, the amount of culture inoculation should be increased.

(6) When the milk has curdled, which should be in 10 or 12 hours, mix the two lots thoroughly by churning or stirring together, bottle, and put on ice to check the acid formation.

BIBLIOGRAPHY.

The following bibliography, while not exhaustive, contains the more important papers on fermented milks and will be useful to those who care to take up this question in more detail:

1. ARNOLD, R.
1899. Contribution à l'étude des laits fermentés. *Le leben*. [Thèse. 44 p. Montpellier.] Abstract in Koch's Jahresbericht über die Fortschritte in der Lehre von den Gährungs-Organismen, vol. 10, p. 226. Leipzig.
2. BALDWIN, HELEN.
1909. Observations on the influence of lactic acid ferments upon intestinal putrefaction in a healthy individual. *Journal of Biological Chemistry*, vol. 7, no. 1, p. 37-48. Baltimore.
3. BELONOVSKY, J.
1907. Influence du ferment lactique sur la flore des excréments des souris. *Annales de l'Institut Pasteur*, vol. 21, no. 12, p. 991-1004. Paris.
1908. Abstract in *Centralblatt für Bakteriologie, Parasitenkunde und Infektionskrankheiten*. Abt. 2, vol. 21, no. 13/14, p. 431. Jena.
4. BERTRAND, GABRIEL, and DUCHAËK, F.
1909. Action du ferment bulgare sur les principaux sucres. *Annales de l'Institut Pasteur*, vol. 23, no. 5, p. 402-414. Paris.
5. BERTRAND, GABRIEL, and WIESWEILLER, GUSTAVE.
1906. Action du ferment bulgare sur le lait. *Annales de l'Institut Pasteur*, vol. 20, no. 12, p. 977-990. Paris.
6. BEYERINCK, MARTINUS W.
1889. Sur le kéfir. *Archives Néerlandaises des Sciences Exactes et Naturelles*, vol. 23 (old series), p. 428-444. The Hague.
7. BIEL, J.
1874. Untersuchungen über den Kumys und den Stoffwechsel während der Kumyskur. 51 p. Vienna.
8. BRUDZINSKI, J.
1900. Ueber das Auftreten von proteus vulgaris in Säuglingsstühlen nebst einem Versuch der Therapie mittelst darreichung von Bacterien-culturen. *Jahrbuch für Kinderheilkunde und Physische Erziehung*, vol. 52, no. 3, p. 469-484. Berlin.
9. BRUHN, F.
1906. Kefir und Kumyss. *Zeitschrift für Fleisch und Milchhygiene*, vol. 16, no. 6, p. 181-184. Berlin.
10. BRUSH, EDWARD F.
Kumyss. 20 p. [n. d., n. p.]
11. CARRICK, GEORGE L.
1881. Koumiss or fermented mare's milk and its uses in the treatment and cure of pulmonary consumption and other wasting diseases. With an appendix on the best methods of fermenting cow's milk. 294 p. Edinburgh and London.
12. COHENDY, MICHEL.
1906. Essais d'acclimatation microbienne persistante dans la cavité intestinale. Délimitation du siège probable de cette acclimatation. *Comptes Rendus Hebdomadaires des Séances de la Société de Biologie*, vol. 60 (année 58, tome 1), no. 7, p. 364-366. Paris.

13. COHENDY, MICHEL.

1906. Description d'un ferment lactique puissant capable de s'acclimater dans l'intestin de l'homme. Comptes Rendus de la Société de Biologie, vol. 60 (année 58, tome 1), no. 11, p. 558-560. Paris.

14. COHENDY, MICHEL.

1906. De la désinfection intestinale obtenue, sans régime special, par l'acclimatation d'un ferment lactique dans le gros intestin. Comptes Rendus Hebdomadaires des Séances de la Société de Biologie, vol. 60 (année 58, tome 1), no. 13, p. 602-604. Paris.

15. COHENDY, MICHEL.

1906. Essai de traitement de l'entérite muco-membraneuse aiguë par l'acclimatation d'un ferment lactique dans le gros intestin. Comptes Rendus Hebdomadaires des Séances de la Société de Biologie, vol. 60 (année 59, tome 1), no. 18, p. 872-874. Paris.

16. CONRADI, HEINRICH, and KURPJUWEIT, O.

1905. Ueber spontane Wachstumshemmung der Bakterien infolge Selbstvergiftung. Münchener Medizinische Wochenschrift, vol. 52, no. 37, p. 1761-1764. Munich.

17. CONRADI, HEINRICH, and KURPJUWEIT, O.

1905. Ueber die Bedeutung der bakteriellen Hemmungsstoffe für die Physiologie und Pathologie des Darms. Münchener Medizinische Wochenschrift, vol. 52, no. 45, p. 2164-2168, and no. 46, pp. 2228-2232. Munich.

18. CRITHARI, C.

1908. Étude sur la symbiose du bacille bulgare et du bacille butyrique. Comptes Rendus Hebdomadaires des Séances de la Société de Biologie, vol. 64, no. 16, p. 818. Paris.

19. DISTASO, A. and SCHILLER, J.

1914. Sur l'acclimatation dans le gros intestin de microbes étrangers à la flore intestinale. Comptes Rendus Hebdomadaires des Séances de la Société de Biologie, vol. 76, no. 6, p. 243-244.

20. DOANE, C. F. and ELDREDGE, E. E.

1915. The use of *Bacillus bulgaricus* in starters for making Swiss or Emmental cheese. U. S. Department of Agriculture Bulletin 148.

21. DÜGGELI, MAX.

1905. Bakteriologische Untersuchungen über das armenische Mazun. Centralblatt für Bakteriologie, Parasitenkunde und Infektionskrankheiten, Abt. 2, vol. 15, nos. 19/20, p. 577-600. Jena.

22. EMMERLING, OSKAR.

1898. Ueber armenisches Mazun. Centralblatt für Bakteriologie, Parasitenkunde und Infektionskrankheiten. Abt. 2, vol. 4, no. 10, p. 418-421. Jena.

23. ENGEL, FRIEDRICH.

1908. Yoghurt oder saure Milch und ihre Fabrikations-Methoden. Molkerei-Zeitung, vol. 22, no. 51, p. 1461-1463. Hildesheim.

24. ESAULOW, N.

1895. Bakteriologische und chemische Untersuchung des Kefir. [Diss. Moskau Pharmazeutische Zeitschrift für Russland, vol. 34, p. 232.] Abstract in Koch's Jahresbericht über die Fortschritte in der Lehre von den Gährungs-Organismen, vol. 6, p. 222.

25. FREUDENREICH, EDWARD VON.

1897. Recherches bacteriologiques sur le kéfir. Annales de Micrographie, vol. 9, p. 5-33. Paris.

26. FUHRMANN, FRANZ.
1907. Über Yoghurt. Zeitschrift für Untersuchung der Nahrungs- und Genussmittel, vol. 13, no. 10, p. 598-604. Berlin.
27. GEBHARD, FRITZ.
1884. Ueber Kephir, seine Bereitung und therapeutische Verwerthung. Inaugural dissertation. Würzburg.
28. GRAABE, K. B.
1909. Yoghurt. Mælkeritidende, vol. 22, no. 1, p. 8-12. Odense.
1909. Abstract in Milch-Zeitung, vol. 38, no. 10, p. 112-113. Leipzig.
29. GRIGOROFF, STAMEN.
1905. Étude sur un lait fermenté comestible. Le Kissélomléko de Bulgarie. Revue Médicale de la Suisse Romande année 25, no. 10, p. 714-721. Geneva.
30. HALLION, L.
1900. Le kéfir. Presse Médicale, vol. 8, no. 43, p. 265-267. Paris.
31. HAMMARSTEN, OLOF.
1888. Untersuchungen von Kefir. [Upsala Läkareförs. Förhand, vol. 21, p. 1-32. 1886.] Abstract in Biedermann's Central-Blatt für Agrikulturchemie und Rationellen Landwirtschafts-Betrieb. vol. 17, p. 413-417. Leipzig.
32. HASTINGS, EDWARD G.
1908. A preliminary note on a group of lactic-acid bacteria not previously described in America. Science, vol. 28, no. 723, p. 656. New York.
33. HASTINGS, EDWARD G., and HAMMER, B. W.
1909. The occurrence and distribution of organisms similar to the *B. bulgaricus* of yoghurt. Centralblatt für Bakteriologie, Parasitenkunde und Infektionskrankheiten, Abt. 2, vol. 25, no. 14/18, p. 419-426. Jena.
34. HAYEM, GEORGES.
1904. Indications thérapeutiques du képhir. Presse Médicale, no. 78, p. 617-620. Paris.
35. HEINEMANN, PAUL G.
1909. Lactic acid as an agent to reduce intestinal putrefaction. Journal of the American Medical Association, vol. 52, no. 5, p. 372-376. Chicago.
36. HEINEMANN, PAUL G., and HEFFERAN, MARY.
1909. A study of *Bacillus bulgaricus*. Journal of Infectious Diseases, vol. 6, no. 3, p. 304-318. Chicago.
37. HENNEBERG, WILLIAM.
1908. Yoghurt. Milch Zeitung, vol. 37, no. 43, p. 506-509. Leipzig.
38. HERSCHELL, GEORGE A.
1908. On the use of selected lactic acid bacilli and soured milk in the treatment of some forms of chronic ill-health. Lancet, vol. 175, no. 4432, p. 371-374. London.
39. HERSCHELL, GEORGE A.
1909. Soured milk and pure cultures of lactic acid bacilli in the treatment of disease. 32 p. London.
40. HERTER, CHRISTIAN A.
1897. On certain relations between bacterial activity in the intestine and the indican of the urine. British Medical Journal, no. 1930, p. 1847-1849. London.
41. HERTER, CHRISTIAN A.
1909. On the therapeutic action of fermented milk. Popular Science Monthly, vol. 74, no. 1, p. 31-42. New York.

42. HERTER, CHRISTIAN A., and KENDALL, A. I.
1908. An observation on the fate of *B. bulgaricus* (in Bacillac) in the digestive tract of a monkey. Journal of Biological Chemistry, vol. 5, no. 2/3, p. 293-302. Baltimore.
43. HERTER, CHRISTIAN A., and KENDALL, A. I.
1910. The influence of dietary alternations on the types of intestinal flora. Journal of Biological Chemistry, vol. 7, no. 3, p. 203-236. Baltimore.
44. KEFIR.
1908. Pure Products, vol. 4, no. 6, p. 245-248. New York.
45. KERN, EDUARD.
1882. Ueber ein Milchferment des Kaukasus. Botanische Zeitung, vol. 40, no. 16, p. 263-264. Leipzig.
46. KERN, TIBOR.
1909. [Effect of the Yoghurt bacillus (*Bacillus bulgaricus*) on the *Bacterium coli*. Orvosi Hetilap, p. 416. 1909.] Abstract in Zentralblatt für die Gesamte Physiologie und Pathologie des Stoffwechsels, vol. 10 (neue Folge, vol. 4), no. 21, p. 843. Berlin.
47. KLOTZ, MAX.
1908. Zur Bakteriologie des Yoghurts. Centralblatt für Bakteriologie, Parasitenkunde und Infektionskrankheiten, Abt. 2, vol. 21, no. 13/14, p. 392-398. Jena.
48. KLOTZ, MAX.
1908. Ueber Yoghurtmilch als Säuglingsnahrung. Jahrbuch für Kinderheilkunde und Physische Erziehung. III F, Band 67, Ergänzungsheft p. 1-56. Berlin.
1909. Abstract in Centralblatt für Bakteriologie, Parasitenkunde und Infektionskrankheiten, Abt. 2, vol. 22, no. 14/17, p. 437. Jena.
49. KOUTMISS.
1907. Pure Products, vol. 3, no. 12, p. 559-562. New York.
50. KRAKAUER, I.
1898. Nähr- und heilwerth des echten Kefirs in Krankheiten der harnsauren Diatese und anderen Fällen. Wiener Medizinische Presse, vol. 39, no. 4, p. 134-142. Vienna.
51. KRANNHALS, H.
1884. Ueber das kumys-ähnliche Getränk "Kephir" und über den "Kephir"-pilz. Deutsche Archiv für Klinische Medizin, vol. 35, no. 1-2, p. 18-37. Leipzig.
52. LANG, LE ROY.
1913. A method for the improvement of buttermilk from pasteurized cream. University of Illinois Agricultural Experiment Station Circular 166.
53. LEEDS, ALBERT R.
1887. Foods for invalids and infants. New Jersey dairy commissioner, annual report, p. 37-47. Trenton.
54. LERSCH.
1869. Die Kur mit Milch und den daraus Getränken (Molken, Kumys). 90 p. Bonn.
55. LEVA, J.
1908. Zur Beurteilung der Wirkung des Lactobacillus und der Yoghurtmilch. Berliner Klinische Wochenschrift, vol. 45, no. 19, p. 922-924. Berlin.
1908. Abstract in Biochemisches Centralblatt, vol. 7, no. 14, p. 546. Leipzig.

56. LUERSSSEN, ARTUR and KÜHN, M.
1908. Yoghurt, die bulgarische Säuermilch. Centralblatt für Bakteriologie, Parasitenkunde und Infektionskrankheiten, Abt. 2, vol. 20, no. 8/9, p. 234-248. Jena.
57. McFARLANE, ARTHUR E.
1905. Prolonging the prime of life. Metchnikoff's discoveries show that old age may be postponed. McClure's Magazine, vol. 25, no. 5, p. 541-551. New York.
58. METCHNIKOFF, ELIE.
1908. The prolongation of life; optimistic studies. Translated by P. Chalmers Mitchell. New York. Chapter 5.
59. METCHNIKOFF, ELIE.
1909. The utility of lactic microbes with explanation of the author's views on longevity. Century Magazine, vol. 79, no. 1, p. 53-58. New York.
60. MICHELS, JOHN.
1909. Manufacture and marketing of cottage cheese, skim milk, buttermilk, and ice cream. North Carolina Agricultural Experiment Station Bulletin 202. West Raleigh.
61. MORO, ERNST.
1900. Ueber den *Bacillus acidophilus* n. spec. Ein Beitrag zur Kenntniss der normalen Darmbakterien des Säuglings. Jahrbuch für Kinderheilkunde und Physische Erziehung, vol. 52, no. 1, p. 38-55. Berlin.
62. MORRES, W.
1909. Die Bereitung von Yogurt (yoghourt). Milch Zeitung, vol. 38, no. 42, p. 497-498. Leipzig.
63. MÜLLER, WALTER.
1909. Die verschiedenen Arten gegorner Milch. Molkerei-Zeitung, vol. 19, no. 44, p. 517-518. Berlin.
64. NIKOLAIIEWA, E. G.
1908. Die Mikroorganismen der Kefirs. Bulletin du Jardin Impérial Botanique de St. Petersburg, vol. 7, no. 4, p. 121-142.
1909. Abstract in Milchwirtschaftliches Zentralblatt, vol. 5, no. 2, p. 91. Leipzig.
65. NORTH, CHARLES E.
1909. Reports of 300 cases treated with a culture of lactic-acid bacteria. Medical Record, vol. 75, no. 13, p. 505-514. New York.
66. OLSCAHNETZKY, M. A.
1890. Untersuchung über den Stoffwechsel während der Kephyreure. Deutsche Medizinische Wochenschrift, vol. 16, no. 27, p. 589-592. Leipzig.
67. PABST.
1904. Fabrication du lait champagnisé et du kéfir. [L'Agriculture Moderne, 1903.] Abstract in Revue Générale du Lait, vol. 3, no. 7, p. 165-166. Lierre.
68. PIFFARD, HENRY G.
1908. A study of sour milks. New York Medical Journal, vol. 87, no. 1, p. 1-9. New York.
69. PODWYSSOTZKY.
1902. Le képhir. 76 p. Paris.
70. RAHE, ALFRED H.
1915. A study of the so-called implantation of the *Bacillus bulgaricus*. Journal of Infectious Diseases, vol. 16, no. 2, p. 210-220.

71. RETTGER, LEO F.

1915. The influence of milk feeding on mortality and growth, and on the character of the intestinal flora. *Journal of Experimental Medicine*, vol. 21, no. 4, p. 365-388.

72. RIST, EDOUARD, and KHOURY, JOSEPH.

1902. Etudes sur un lait fermenté comestible. Le "leben" d'Egypte. *Annales de l'Institut Pasteur*, vol. 16, no. 1, p. 65-84. Paris.

73. [ROSENTHAL, GEORGES, and CHAZARAIN-WETZEL, P.

1909. Essais de bactériothérapie clinique (Première communication). De l'emploi des ferments lactiques (bacille bulgare et streptocoque lactique) dans le traitement des infections chirurgicales des voies urinaires et de la vessie en particulier. *Bulletin Général de Thérapeutique Médicale*, vol. 158, p. 17. July, 1909.] Abstract in *Biochemisches Centralblatt*, vol. 9, no. 1-2, p. 50. Leipzig.

74. SAMARANI, F.

1909. "Cieddu." [Annuario della Reale Stazione Sperimentale di Caseificio di Lodi. Lodi, 1907, p. 95-98.] Abstract in *Experiment Station Record*, vol. 20, no. 5, p. 477. Washington.

75. SCHIPIN, D.

1900. Ueber den Kumysbacillus. *Centralblatt für Bakteriologie, Parasitenkunde und Infektionskrankheiten*, Abt. 2, vol. 6, no. 23, p. 775-777. Jena.

76. SEVERIN, S. A.

1908. Einige Ergebnisse und Bemerkungen über den sogenannten *Bacillus bulgaricus* und das Milchsäurepräparat "Lactobacilline." *Centralblatt für Bakteriologie, Parasitenkunde und Infektionskrankheiten*, Abt. 2, vol. 22, no. 1/2, p. 3-22. Jena.

77. STANISLAUS, I. V. S.

1908. Kefir and its preparation. *American Journal of Pharmacy*, vol. 80, no. 1, p. 20-26. Philadelphia.

78. STEKHOVEN, J. H.

1891. Schuurmans. *Saccharomyces kefir*. Onderzoekingen gedaan in het Physiologisch Laboratorium der Utrechtsche Hoogeschool, vol. 2, p. 119. Utrecht.

79. THEODOROFF, J.

1886. Historische und experimentelle Studien über den Kephir, Inaugural dissertation. Würzburg.

80. TISSIER, HENRY.

1905. Étude d'une variété d'infection intestinale chez le nourrisson. *Annales de l'Institut Pasteur*, vol. 19, no. 5, p. 273-297. Paris.

81. WEIGMANN, H., and others.

1907. Ueber armenisches Mazun. *Centralblatt für Bakteriologie, Parasitenkunde und Infektionskrankheiten*. Abt. 2, vol. 19, no. 1/3, p. 70-87. Jena.

82. WHITE, BENJAMIN, and AVERY, OSWALD T.

1909. Observations on certain lactic-acid bacteria of the so-called bulgaricus type. *Centralblatt für Bakteriologie, Parasitenkunde und Infektionskrankheiten*. Abt. 2, vol. 25, no. 5/9, p. 161-178. Jena.

PUBLICATIONS OF U. S. DEPARTMENT OF AGRICULTURE RELATING TO MILK.

AVAILABLE FOR FREE DISTRIBUTION.

- Use of Milk as Food. (Farmers' Bulletin 363.)
- Care of Milk and Its Use in the Home. (Farmers' Bulletin 413.)
- Bacteria in Milk. (Farmers' Bulletin 490.)
- Farm Butter Making. (Farmers' Bulletin 541.)
- Clean Milk: Production and Handling. (Farmers' Bulletin 602.)
- Application of Refrigeration to the Handling of Milk. (Department Bulletin 98.)
- Alcohol Test in Relation to Milk. (Department Bulletin 202.)
- Pasteurizing Milk in Bottles and Bottling Hot Milk Pasteurized in Bulk. (Department Bulletin 240.)
- Estimation of Total Solids in Milk by Use of Formulas. (Bureau of Animal Industry Bulletin 134.)
- Influence of Stage of Lactation on Composition and Properties of Milk. (Bureau of Animal Industry Bulletin 155.)
- Chemical Changes Produced in Cow's Milk by Pasteurization. (Bureau of Animal Industry Bulletin 166.)
- Extra Cost of Producing Clean Milk. (Bureau of Animal Industry Circular 170.)
- Plan for Small Dairy House. (Bureau of Animal Industry Circular 195.)
- Control of Bulk Milk in Stores. (Bureau of Animal Industry Circular 217.)

FOR SALE BY THE SUPERINTENDENT OF DOCUMENTS.

- Cost of Pasteurizing Milk and Cream. (Department Bulletin 85.) Price, 5 cents.
- Bacteria of Pasteurized and Unpasteurized Milk Under Laboratory Conditions. (Bureau of Animal Industry Bulletin 73.) Price, 5 cents.
- Bacteriology of Commercially Pasteurized and Raw Market Milk. (Bureau of Animal Industry Bulletin 126.) Price, 15 cents.
- Study of Bacteria Which Survive Pasteurization. (Bureau of Animal Industry Bulletin 161.) Price, 10 cents.
- Some Important Factors in Production of Sanitary Milk. (Bureau of Animal Industry Circular 142.) Price, 5 cents.
- Improved Methods for Production of Market Milk by Ordinary Dairies. (Bureau of Animal Industry Circular 158.) Price, 5 cents.
- Fermented Milk. (Bureau of Animal Industry Circular 171.) Price, 5 cents.
- Pasteurization of Milk. (Bureau of Animal Industry Circular 184.) Price, 5 cents.
- Directions for Home Pasteurization of Milk. (Bureau of Animal Industry Circular 197.) Price, 5 cents.
- Milk and Cream Contests: How to Conduct Them, and How to Prepare Samples for Competition. (Bureau of Animal Industry Circular 205.) Price, 5 cents.

PUBLICATIONS OF U. S. DEPARTMENT OF AGRICULTURE
IND TO MILK

ADDITIONAL COPIES
OF THIS PUBLICATION MAY BE PROCURED FROM
THE SUPERINTENDENT OF DOCUMENTS
GOVERNMENT PRINTING OFFICE

WASHINGTON, D. C.

AT
5 CENTS PER COPY



